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FATIGUE COUNTERMEASURES FOR RAPID DEPLOYMENT: OPERATION PEGASUS

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14. ABSTRACT

The aim of this work was to investigate the effects of slow-release caffeine (SRC) and melatonin (MLT) on sleep and daytime vigilance and SRC or MLT as chronobiotics after a 7-time zone eastbound flight. Three groups of 9 US Air Force reservists each received either five daily doses of SRC (300 mg), four nightly doses of MLT (5 mg) or the appropriate placebo for SRC or MLT in a double-blind, randomized manner. Nighttime sleep was evaluated by polysomnography and daytime vigilance by Multiple Sleep Latency Tests (MSLT) and continuous wrist actigraphy. Cognitive function was assessed by computerized tests from the AGARD-NATO STRES Battery. Attention was also assessed with a symbol cancellation task and the Stroop test and alertness was gauged by a visual analogue scale (VAS). All tests but nighttime sleep and neuroendocrine samples were performed in two blocks, during the AM and PM. Core temperature was continuously measured and safety of treatment was assessed from repeated clinical examinations. Saliva and urine were sampled before the flight in the United States for a baseline (from Day -2 to Day 0) and after the flight in France (from Day 1 to Day 10). Saliva was collected once a day on waking to determine saliva melatonin and cortisol concentrations. In addition, concentrations of caffeine in saliva were determined three times a day and 6-sulphatoxymelatonin in urine also was collected upon awakening.

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This project was a collaborative effort between the Air Force Research Laboratory Brooks AFB, TX and the Institut de Medicine Aerospatiale du Service de Sante des Armees (IMASSA) in July-August 1998 to conduct an operational study of the pineal hormone melatonin and sustained release caffeine as a means to blunt the deleterious effects of fatigue generated by rapid, eastward, transmeridian travel. The Project was code named Pegasus in honor of the mythological horse who knew no restrictions of borders as it soared across ancient skies. As of this writing, March 2003, Dr. French is no longer with the AFRL and can be reached at 139 Nandina Terrace Winter Springs, FL 32708, (407) 482-1292, jfrench1@cfl.rr.com. Mr Whitmore is still with the AFRL. Dr Lagarde is no longer at IMASSA-CERMA but may still be reached through that address via Dr Maurice Beaumont who replaced him. This report is prepared as the final documentation of the effort for the AFRL and may be used in a Technical Report.

ABSTRACT

The aim of this work was to investigate the effects of slow-release caffeine (SRC) and melatonin (MLT) on sleep and daytime vigilance and SRC or MLT as chronobiotics after a 7-time zone eastbound flight. Three groups of 9 US Air Force reservists each received either five daily doses of SRC (300 mg), four nightly doses of MLT (5 mg) or the appropriate placebo for SRC or MLT in a double-blind, randomized manner. Nighttime sleep was evaluated by polysomnography and daytime vigilance by Multiple Sleep Latency Tests (MSLT) and continuous wrist actigraphy. Cognitive function was assessed by computerized tests from the AGARD-NATO STRES Battery. Attention was also assessed with a symbol cancellation task and the Stroop test and alertness was gauged by a visual analogue scale (VAS). All tests but nighttime sleep and neuroendocrine samples were performed in two blocks, during the AM and PM. Core temperature was continuously measured and safety of treatment was assessed from repeated clinical examinations. Saliva and urine were sampled before the flight in the United States for a baseline (from Day -2 to Day 0) and after the flight in France (from Day 1 to Day 10). Saliva was collected once a day on waking to determine saliva melatonin and cortisol concentrations. In addition, concentrations of caffeine in saliva were determined three times a day and 6-sulphatoxymelatonin in urine also was collected upon awakening.

Compared to baseline, a significant increase in Slow Wave Sleep (SWS) on Night 1 and Night 2 (N1-N2) was found with placebo and MLT, and a significant decrease in Rapid Eye Movement (REM) sleep on N1 (placebo) and N1-N3 (MLT). Subjects were sleepier (MSLT) on Day 1- Day 6 (D1-D6) for placebo and D1-D3 for MLT than during baseline. SRC exerted stimulating effects on vigilance but also on sleep until the last drug day. Compared to placebo and the MLT groups the SRC subjects were more vigilant from the onset ($p<0.001$) to the end of the first day in France (SD) ($p< 0.0001$). The SRC group had better cognitive function and alertness during SD, as shown by Stroop's test ($p<0.048$) than placebo or MLT.

SRC reduces daytime sleepiness following jet-lag with some alerting effects on recovery sleep while with MLT, daytime vigilance was not supported although sleep was improved. From Day 3 to Day 5 post-flight, saliva melatonin concentrations were significantly different from baseline values in the placebo group only. As expected, during treatment with melatonin, the mean urinary 6-sulphatoxymelatonin concentration in the melatonin group was more than twice as high

as in the two other groups. In the SRC and the MLT groups, mean saliva cortisol concentrations were significantly lower than baseline from day 2 to day 5, whereas the placebo group had a mean saliva cortisol concentration significantly lower than the baseline value from Day 2 to Day 9. These results suggest that administration of SRC, as well as of MLT allows a faster resynchronization of hormonal rhythms during the 4 days following an eastbound flight.

INTRODUCTION

The circadian phase disruption attributable to rapid transmeridian travel commonly called "jet-lag" is an unpleasant malaise that lasts for several days after relocation. It is experienced by millions of military, business, government and other transcontinental passengers each year, as well as by the crews manning the aircraft transporting them. At pathophysiological levels, the 'biological clock', set at the country of origin, is out of synchrony with the environmental cues to which it is exposed at the country of destination. Additionally, the various circadian rhythms do not all resynchronize at the same speed and can be out of synchrony in relation to each other. The 'biological clock' refers to a series of neurohormonal systems which act to time the circadian oscillation of physiological events. The most important environmental cue (zeitgeber) to maintain (entrain) this fluctuation is the solar light-dark cycle.

For the military, jet lag means a reduction in alertness and poorer sleep until crews can re-entrain the rhythms to their new local environment. Other clinical manifestations can be associated with it, such as a sensation of asthenia, irritability, diminished cognitive performance, anxiety, the appearance of depressive phenomena and digestive disorders. These symptoms appear after a flight crossing four or more time zones and are all the more pronounced the greater the number of zones crossed. The direction of the flight is significant: an eastward flight, which demands an advance in the sleep phase, is less well tolerated than a westward flight, which demands a delay in phase.

Treatments that make it possible to attenuate jet-lag and accelerate the resynchronization of biological rhythms, especially as far as the sleep-wake cycle is concerned, are increasingly more important as more commercial travelers make use of transcontinental missions. Similarly, the military depends on rapidly deployed troops being as vigilant on duty and as well-rested during crew rest as possible. Gradually modifying bedtime before departure is not advisable as this is restricting and can even induce symptoms of jet-lag before the time zone difference. Sleep in-flight is advisable but, in practice, inadequate (Ferrer, et al, 1995). Use of bright lights require long hours of exposure prior to a transmeridian flight at times of day that would be optimal for sleeping, further compounding the sleep deprivation that jet lag can cause (Lebegue, et al. 1995).

Hypnotic drugs of the benzodiazepine category have been studied in relation to the symptomatic treatment of jet-lag: their value lies in the fact that they induce sleep and a better nights sleep is achieved but, generally, an alteration in psychomotor performance during daytime is observed, as is an increase in daytime drowsiness and the risk of anterograde amnesia produced by this class of drugs (Donaldson, et al., 1991; Turek, et al., 1988). Contemporary interest has focused on the pineal hormone melatonin as a means to pre-shift individuals prior to travel with minimum disruption in lifestyle. Evidence suggests that this is a promising use for melatonin. For

example, one of the suggested results of melatonin administration is to re-synchronize the sleep-wake cycle (Sack, et al. 1997). As well, melatonin is known to have receptor sites on the suprachiasmatic nucleus (SCN), currently considered to be the primary circadian oscillator (Weaver, et al., 1993). Alterations of SCN activity are known to temporally effect circadian phase (McArthur, et al., 1991). Recently a phase-response curve for melatonin was demonstrated showing that, administered in the late afternoon, melatonin could advance circadian phase, and, administered in the early morning, caused a phase delay (Lewy, et al., 1992). It has been successfully used to treat disorders of circadian phase and re-establish normal rhythmicity (Dawson, et al., 1996). These pioneering efforts suggest that melatonin may be a rapid and convenient means to alter the circadian system prior to travel so that one arrives nearly in-phase with the destination time (Arendt, et al., 1997; Petrie, et al. 1993). However, studies of the effects of melatonin on transmeridian travel are few, in part because of the large number of subjects required and the corresponding expense of transmeridian travel. Additionally, the metrics used are often nothing more than subjective reports.

Melatonin was selected for study as a potential countermeasures on the basis that it may have the ability to rapidly resynchronize the circadian system to a new setting. Caffeine also was selected because there has recently been a suggestion that as an adenosine antagonist, it may affect pinealocytes and inhibit melatonin production. Moreover, another study of sleep deprivation for 48 h has also highlighted improved alertness and performance using caffeine (200 mg at 8 p.m. and 2 a.m.) and found a fall and delay in melatonin synthesis as well as an attenuation in temperature decline during the night-time period (Wright, et al., 1997). This suggested a mechanism whereby caffeine might be used to alter circadian phase. Of course it is well known that caffeine has positive effects on sleep deprivation effects and potent alerting properties as a psychostimulant, however very few of these studies have examined chronic caffeine administration (Lieberman,et al, 1992). Caffeine, then, was administered to inhibit melatonin production during the subjective night until resynchronization occurred in France and to blunt the fatigue associated with jet lag. Slow release caffeine dosed at 300 mg produces a kinetic rate that is appropriate for use as an effective treatment for fatigue (Nestles Corporation; Switzerland, 1996). Concentrations obtained 24 hours after administration are too low over a short period of repeated doses to cause an accumulation of caffeine that is likely to have an adverse effect.

Slow release caffeine (SRC) is a new formulation of caffeine and, at the single dose of 300 mg, has been demonstrated to have positive effects on alertness and performance over 9-13 h without any major side-effects (Lagarde, et al., 2000). Indeed, the release of caffeine in SRC makes it possible to reach plasmatic plateau within approximately 4 h and to remain at this level for 4-6 h, without overshooting the threshold for untoward side-effects. These effects are shown in Figure 1. The beneficial effects of SRC have been confirmed across a 36-h sleep deprivation (SD) period with a daily dose of 600 mg (Patat, et al., 2000). To summarize, a single daily dose of 300 mg of SRC seems to be superior than repeated moderate or single larger doses of caffeine to enhance performance and alertness for a period of SD longer than 24 h.

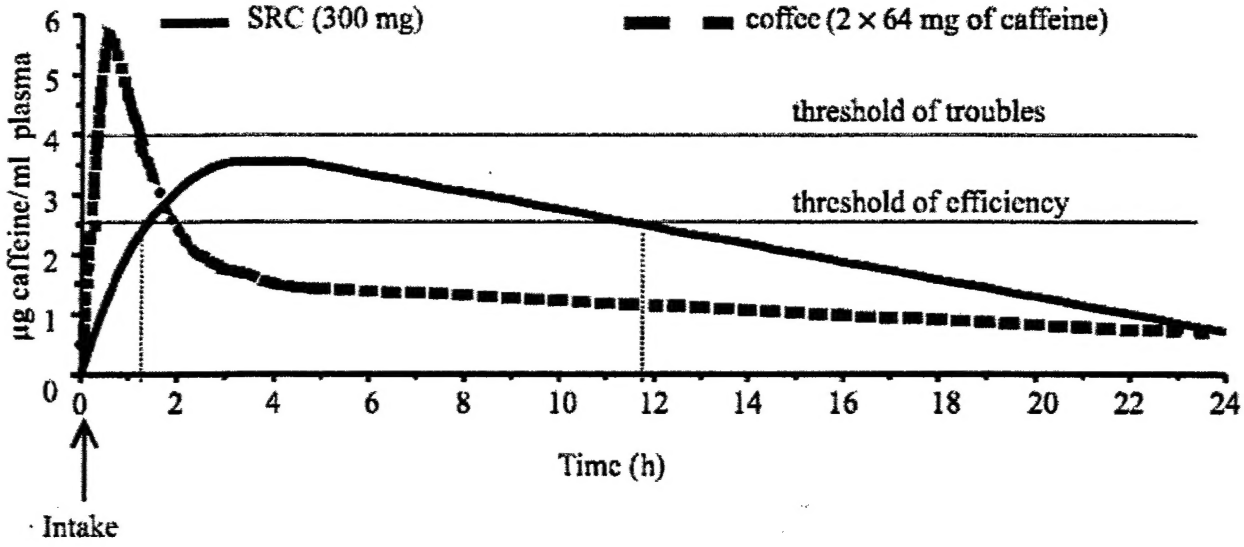

Figure 1. Comparison of Slow Release Caffeine capsule and a normal dose (128 mg caffeine) of coffee. The threshold of troubles is where adverse side effects, diuresis, GI upset, trembling, occur well above the alerting (efficiency) properties (Lagarde 2000).

Melatonin (MLT) and caffeine (SRC) were also selected for this study because neither is an exotic drug and both would have a chance of being fielded in the near future if efficacy could be shown.

It is important to point out that this study would have cost far more if done by contractor or by academicians. As it was, the AF Reservists were able to use their annual tours to defray the individual costs (for board about $440 per person; meals and transportation were provided free). The US flight crew that flew the investigators, subjects and their equipment to the French lab satisfied a training requirement and obtained a rare opportunity to land in France. The French Air Force provided housing, food and the return flight). It would be difficult to imagine a reservist going on annual tour even in the US for 12 days for less cost.

METHODS:

This double blind, randomized, placebo-controlled study was conducted on 27 healthy volunteers from a US Air Force Reserve Unit that was representative of the US population (18 males and 9 females; 15 Caucasians, 9 Hispanics and 3 Afro-Americans; age: 35.3 ± 8.1 yr. (age range: 19-47 yr.); weight: 77.6 ± 15.8 kg; height: 170 ± 10 cm), as requested by the Institutional review board for the USAF. All underwent a medical evaluation before participation. They were nonsmokers with no history of sleep disorders. Horne and Ostberg's (1976) questionnaire showed that they were neither morning nor evening types with a sleep duration ranging from 6.5 to 7.5 hours. They had not experienced a transmeridian flight in the two months before their enrollment in this study. They did not consume large amounts of xanthine-based beverages on a regular basis (coffee, tea and cola: equivalent to less than 3 cups a day), nor had they taken psychotropic drugs or melatonin in the three months prior to the study. They abstained from drinking alcohol or caffeine-containing beverages during the experiment and this commitment

was strictly controlled by the experimenters. They gave informed written consent prior to participation. The study and subject conditions were approved by the Human Use in Experimentation Committee of Brooks AFB (ACHE#95-01A) and was further approved by the Human Ethics Committees of the Robert Ballanger Hospital, Aulnay sous Bois of France.

The 27 subjects were allocated randomly into three parallel groups, each containing 3 women and 6 men, to be administered either 300-mg slow release caffeine (SRC), 5-mg melatonin (MLT) or a placebo (PBO). SRC was administered from D1 to D5 at 0800, the synthetic MLT was administered on D-1 (1700), D0 (1600) and from D1 to D3 (2300), and the appropriate placebo (PBO) was administered in the same schedules to everyone not getting the active compound (Figure 2). The MLT intake schedule before, during and after the transcontinental flight corresponded to normal bedtime in France. MLT with a degree of purity close to 100% was provided to Nestec Research Center Research Center (Lausanne, Switzerland) by Helsinn Chemicals SA (via Industria, Biasca, Switzerland) and SRC and PBO (lactose) by Nestec directly. MLT, SRC and PBO were packaged in gelatin capsules by Nestec according to standard double-blind procedures. Nestec has a long tradition of making both caffeine and melatonin suitable for sale as foodstuffs in Europe and the US.

All of the capsules shared the same appearance to maintain a blinded dosing regimen. The code for the compounds was on record with the Brooks AFB flight surgeon's office, the IMASSA flight surgeon's office and with the PI and Co-PI of the study in the event a medical emergency made it necessary to decipher the code. The capsules were swallowed with 100 ml mineral water (Perrier). They were taken in a standing position under the supervision of an investigator. The time at which the dose was taken and the initials of the person administering the drug were written down in the observation booklet. All compounds were stored at ambient temperature (between 15 and 25°C).

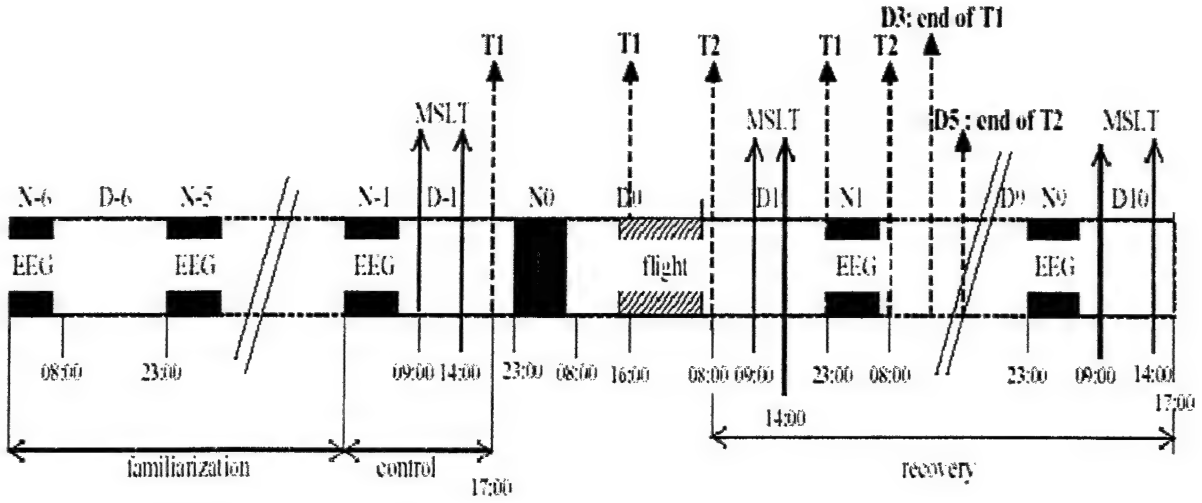

Figure 2. The timing of events in the study starting with Night -6 (N-6) to Day 10 (D10). T1 (treatment 1: Melatonin): for D-1 (1700, US time), D0 (1600, US time) and D1-D3 (2300, French time): 5-mg melatonin for MLT group and placebo for SRC and PBO groups; T2 (treatment 2: caffeine): for D1-D5 (0800, French time): 300-mg slow release caffeine for SRC group and placebo for MLT and PBO groups.

The subjects trained at Brooks AFB in San Antonio, Texas, USA over six Days (D-6 – D-1) to synchronize circadian rhythms. During the first five days, they were familiarized with the procedures and the experimental tests and measurements, including the nocturnal EEG on 12 randomly selected individuals. The capability for recording nocturnal EEG on medilogs was only available for 12 subjects. Baseline data for performance and EEG were obtained during the last night and day (N-1, D-1) to avoid the first night effect (Agnew, Webb, and Williams, 1966).

The flight was scheduled on D0, 1500 (US time) for a 7-time zone eastbound flight to Mont de Marsan, France. According to the test protocol, the subjects were prohibited from sleeping during the flight. They played cards or read and had to complete sleep logs and questionnaires at regular intervals; their state of wakefulness was checked subjectively by the experimenters. The arrival was on D1, 0600 (French time) and recovery lasted 10 days (D1-D10) and 9 nights (N1-N9). Before and after the flight, the daily routine was the same for all subjects: wake up at 0700 (beginning of daylight exposure), breakfast at 0800, morning tests between 0900 and 1200, lunch between 1230 and 1330, evening tests between 1400 and 1700, light muscular activity (walking in the woods) between 1730 and 1830, dinner at 1930, fitting of EEG electrodes from 2000, leisure (reading, games) until bedtime at 2300 (lights off). Light exposure (intensity and duration) was identical for all subjects throughout the experiment to avoid any bias related to a shift in circadian rhythms. None of the subjects slept, ate, or drank or performed unsupervised activity outside the authorized periods.

In order to estimate the resynchronization of melatonin secretion, the nightly urinary excretion of 6-sulphatoxy melatonin, the main metabolite of melatonin, was measured in urine specimens collected at rising from D-2 to D10. Moreover, salivary samples of melatonin and cortisol were collected at 0730 and measured from D-2 to D10.

Body temperature resynchronization was estimated from oral and rectal temperature samples. Buccal temperature readings were taken every two hours from 7 a.m. to 11 p.m. (from D-2 to D10) and recorded on the subject log sheet. As well, uninterrupted nocturnal rectal temperature readings (performed on the twelve subjects undergoing the sleep EEG, i.e. polysomnography) made it possible to monitor body temperature resynchronization. These same 12 subjects were used for sleep EEG and temperature recordings throughout the study. No other invasive measures were taken. Temperature peaks were expected about 1600 France time in adjusted individuals in France and temperature nadirs were expected at 0900 in unadjusted individuals in France.

Measurements

Sleep

Baseline and recovery sleep architecture was assessed from standard polysomnographic recordings including electroencephalography (C3/Cz, O1/O2, referenced to an A1 ground electrode on the mastoid apophysis), electrooculography of each eye (oblique and horizontal derivations), chin electromyography. Polysomnography electric signals were sampled, amplified and stored using a portable Medilog 9000-2 (Oxford Medical Instruments, Abingdon, England) from 2300 to 0630 during the baseline (N-1) and recovery (N1-N9) nights (see Fig 2). EEG recordings were scored in 30-s epochs according to standard criteria (Rechtschaffen, and Kales, 1968) by a researcher who was unaware of the medication taken (SRC, MLT or placebo). We calculated for each night: sleep period time (SPT: time from falling asleep to last awakening), total sleep time (TST: difference between SPT and wakefulness after sleep onset (WASO), sleep efficiency index (SEI= TST/TIB, with TIB= time in bed) and sleep onset latency (SOL: time from lights out to 1st episode of stage 2). We also measured SWS and REM latencies (time from 1st stage 2 to 1st epochs of stage 3 and REM sleep, respectively). Each stage of sleep was analyzed by measuring total duration and percent of TST; the number of SWS and REM sleep episodes were also counted. The REM sleep stability index (REM SSI) was defined as the percent of actual REM sleep within REM sleep episodes. Because the female subjects refused to wear the electrocaps throughout the night, the polysomnographic data were only available for four males in each experimental group. However, qualitative and quantitative aspects of sleep were evaluated in male and female subjects from sleep logs completed after wake-up from D1 to D10.

Daytime vigilance

Over baseline and recovery periods, sleepiness was assessed from Multiple Sleep Latencies Tests (MSLT) at 0900 and 1400, corresponding to the hyper and hypovigilance periods defined by Lavie (1986), and from continuous wrist actigraphy. Vigilance and mood were evaluated from

Bond and Lader's (1974) visual analog scales (VAS) during baseline and recovery periods, and also during the flight, 1, 3, and 5 hours following drug intake (1600). These objective and subjective measurements were taken from all subjects.

The MSLT is a standard physiological tool for quantifying sleepiness, and is based on the assumption that sleepiness is a physiological need state that leads to an increased tendency to fall asleep (Carskadon, Dement, Mitler, and Roth T, 1998) . The latency to fall asleep (sleep latency) was measured by polysomnography in all subjects, while lying with eyes shut in a quiet, dark room. Subjects were instructed to allow themselves to fall asleep or not to resist falling asleep. The test was stopped if subjects did not fall asleep within 20 min after the start of the test period (lights off) or after either three consecutive 20-s epochs of stage 1 or one first 20-s epoch of stage 2 sleep or of REM sleep.

Continuous wrist actigraphy was employed to evaluate vigilance (Lockley, Skene, and Arendt, 1999;. Monk, Buysse, and Rose, 1999; Reid, and Dawson, 1999). Subjects wore a piezoelectric accelerometer (Gaehwiler Electronic, sensitivity: 0.1 G, sampling rate: 8 Hz, band-pass filter: 0.25-3 Hz, data acquisition period: 15 s) on their non-dominant wrist throughout the experiment. The number of movements with a force greater than 0.1 G was counted over each hour for 24 hours.

Methods of analysis

Saliva melatonin, cortisol and urinary a-MT6 s concentrations were measured in the Department of Cellular and Structural Biology of the University of Texas Health Science Center in San Antonio (TX, USA). Caffeine assays were carried out by the Centre de Recherches Nestlé in Lausanne (Switzerland).

Melatonin

Saliva melatonin concentration was quantified using radioimmunoassay (RIA), employing a Stockgrand antibody and reagents (Guilford, Surrey, UK). Briefly, 500 µl of saliva were incubated in a tricine buffer (0.1 mol·l-1; pH 5.5) with 200 µl of sheep antiserum. A volume of 100 µl of tritiated melatonin was added to the mixture. Incubation was for 18 h at +4°C. Then the bound and free fractions were separated using 500 µl of a dextran-coated charcoal suspension. The free melatonin fraction was precipitated with charcoal by centrifugation (1,500 rpm; 15 min at 4°C) after 15 min of incubation at 4°C. The radioactivity of the antibody-bound fraction in the supernatant was then counted in a scintillation counter. An 8 concentration calibration curve (0-500 pg·ml-1) was constructed at the same time from standard melatonin. In order to avoid contamination, precautions were taken as regards glassware cleanliness and distilled water quality. All the measurements were made in duplicate.

Cortisol

Cortisol was assayed by solid-phase RIA according to the Coat-a-Count method (Diagnostic Products Corporation). A volume of 1 ml of 125I-labelled cortisol was added to each saliva sample (200 µl) and kept for 3 h at room temperature. Because the antibody is immobilized by attachment to the wall of a polypropylene tube, simply decanting the supernatant was sufficient to terminate the competition and to isolate the antibody-bound fraction of the radio-labeled

cortisol. The tube was then counted in a radiation counter for 1 min, the values obtained then being compared to a 6 point calibration curve (0-5 µg·dl-1) to determine the concentration of cortisol present in the sample.

6-Sulphatoxymelatonin

The compound 6-sulphatoxymelatonin (a-MT6 s) was quantified by RIA (Stockgrand Ltd) after the urine had been diluted 1:250 with tricine buffer (0.1 mol·l-1; pH 5.5). A volume of 500 µl of the diluted sample was incubated with 200 µl of anti a-MT6 s sheep antiserum and 100 µl of 125I-labelled a-MT6 s for 15-18 h at 4°C. Separation of bound and free fractions was obtained by the addition of 100 µl of dextran-coated charcoal, incubation for 15 min at +4°C under continuous agitation, followed by centrifugation (3,500 rpm; 15 min at 4°C). The supernatant was immediately discarded by inverting the tube on a paper towel. The pellet was counted in a - radiation counter for 1 min. A 9 point dose-response curve (0-50 ng·ml-1) was constructed using a-MT6 s diluted in free endogenous a-MT6 s urine (obtained by passing it over active charcoal). Standards and samples were processed in the same way in duplicates.

Caffeine

A high performance liquid chromatography (HPLC) technique after liquid-liquid extraction from saliva was used. A volume of 0.30 ml of saliva sample was added to 30 µl of internal standard (7-ethyl theophylline; 0.10 mg·ml-1) and 6 ml of chloroform-isopropanol 9:1 (v/v). The mixture was then stirred for 30 s and the organic phase separated by centrifugation (2,000 rpm; 5 min). This phase was evaporated at 40°C (under nitrogen) and the residue diluted in 500 µl of 0.05% acetic acid. A volume of 100 µl of this solution was injected into the HPLC system. The separation of methylxanthines was performed on a reverse-phase column (Nucleosil 5-C18; Macherey-Nagel) using an isocratic elution (0.05% acetic acid and 35% methanol in water; flow rate 1 ml·min-1.). Their detection required an ultraviolet detector with its wavelength set at 272 nm. A 5 point calibration curve was constructed from 1 to 20 µg·ml-1 (1, 2.5, 5, 10, and 20 µg·ml-1). A linear response was observed over this range and the quantification limit was 500 µg·ml-1 of saliva.

Statistical analysis

Sleep and vigilance data were analyzed separately and compared by two-way ANOVA (drug: SRC, MLT, placebo; period of time: recovery vs baseline) with repeated measurements over time. Except for the EEG data, gender was also taken into account as a covariant factor. The level of significance (p) was set at 0.05. The Newman-Keuls test was employed for the *posthoc* comparisons.

RESULTS

Baseline sleep

Since analysis of the baseline polysomnographic recordings did not reveal any first night effect, the subjects were allocated at random into three groups (SRC, MLT and placebo, see methods). There were no significant differences in sleep parameters between the subjects of these three groups.

Upon comparing data measured during recovery to baseline within each drug group, no subject slept longer than on baseline night (N-1). TST, SPT and WASO did not change significantly throughout the experiment in all groups, except SPT which fell by ~30 min in the SRC group on N4 ($p < 0.05$). However, compared to baseline, SRC subjects showed an increase in WASO by about 40 and 20 min on N3 and N4, then a drop of about 40 min in N5, although these changes did not reach significance. Since TST and TIB were constant for all subjects, SEI did not vary throughout recovery in any of the groups. Compared to baseline, sleep latency, non-REM and REM sleep measured during recovery depended on treatment.

Sleep latency (SOL) results showed that placebo subjects fell asleep earlier on N1 (SOL: - 8 min, $p < 0.05$), but they fell asleep later on N4, N5 and N6 (SOL: + 18, + 30 and + 28 min, respectively; $p < 0.05$). SOL of SRC subjects was not shortened on N1, but it was lengthened at the end of the treatment (N4: + 29 min, $p < 0.05$; N5: + 11 min, $p < 0.05$); by contrast, SOL of MLT subjects did not change throughout the entire recovery period.

Non-REM sleep results compared to baseline showed that SWS was longer in subjects receiving placebo and to a lesser degree in those receiving MLT on N1 (PBO: + 53 min , MLT: + 33 min, $p < 0.05$), N2 (PBO: + 69 min, MLT: + 28 min, $p < 0.05$) and N5 for PBO subjects only (+ 37 min, $p < 0.05$). SWS also appeared significantly earlier (SWS latency decreased) in all these subjects on these nights. This rebound in SWS was observed at the expense of stage 2, which decreased in PBO subjects on N2, N3 and N5 by 86, 65 and 44 min ($p < 0.05$) and also decreased under MLT on N1, N2 and N4 (- 18, -20, -31 min, $p < 0.05$). In contrast, in the subjects receiving SRC, SWS did not occur earlier (it was even delayed in N4 by 17 min, $p < 0.05$) and the rebound in SWS was postponed to N6, the night following the end of drug administration (SWS: + ~21 min, $p < 0.05$).

REM sleep data revealed that the rebound of SWS in PBO and MLT subjects was observed at the expense of REM sleep which decreased by 29 min in PBO subjects on N1 ($p < 0.05$) and by 24, 15 and 25 min; under MLT on N1, N2, N3 ($p < 0.05$). The expected rebound in REM sleep was observed on N2 in PBO subjects only (+ 22 min, $p < 0.05$); it also came earlier than in baseline conditions (REM sleep latency: - 49 min; $p < 0.05$). By contrast, no modification of REM sleep was observed under SRC throughout the recovery period.

On N1, compared with PBO, stage 1 was increased under SRC (+ 8 min, $p < 0.05$) and decreased under MLT (- 8 min, $p < 0.05$). Stage 2 under MLT was longer than in the other subjects on N3 ($p < 0.05$). The rebound of SWS on N2 was shorter in MLT than in PBO subjects ($p < 0.05$).

Subjective aspects of sleep

Sleep logs also identified few differences between the drug groups on any given night. On N1, the SRC subjects woke up earlier, had a shortened sleep duration and complained of more awakenings than did the MLT group ($p < 0.05$), whereas MLT subjects fell asleep earlier and slept longer than did the PBO group ($p < 0.05$). Waking from sleep for the SRC group was more difficult than for the PBO group on N5 ($p < 0.05$), but sleep under SRC was longer and better than in the MLT group on N6 ($p< 0.05$).

Comparisons between recovery nights with baseline data within each drug group did not show the differences observed with the EEG recordings. In PBO subjects, TST was decreased on N4 ($p< 0.031$). Under SRC, TST was also decreased on N4 ($p< 0.039$), but increased on N6 ($p< 0.027$) at the end of the treatment, whereas the quantity of sleep and the quality of REM and wake up time seemed to be insufficient or impaired on N2 ($p< 0.011$), N3 ($p< 0.029$) and N5 ($p< 0.034$). Under MLT, TST appeared shorter on N1 ($p< 0.022$), quality of sleep better on N1 and N2 ($p< 0.013$ and 0.034, respectively) with a lower duration of sleep on N2 ($p< 0.034$) and higher quality of wake up on N2 ($p< 0.034$). Sleep logs showed a gender difference during the first five days of recovery: for all treatment groups, sleep difficulties were statistically more marked in the female than in the male subjects.

Objective measures of vigilance

Sleep latencies obtained by MSLT over baseline and recovery periods are shown in Figure 3. The overall circadian rhythm of vigilance was maintained in all subjects throughout the experiment. Neither objective nor subjective measurements of vigilance showed any significant gender effect.

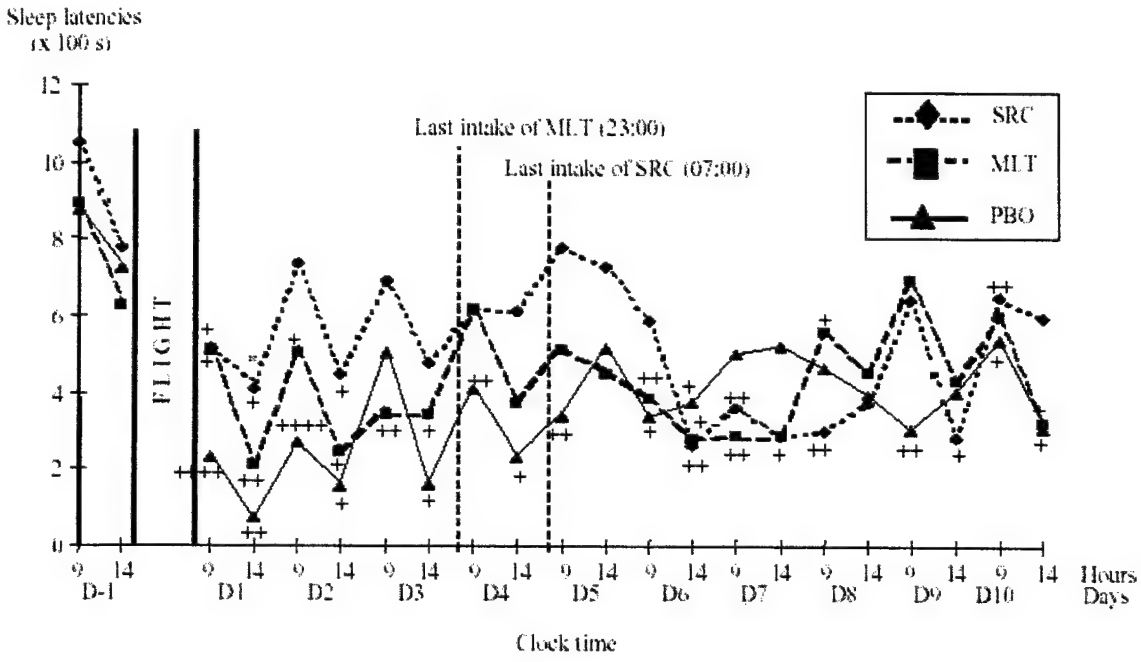

Figure 3. Sleepiness assessed by measurements of sleep onset latencies (MSLT) in PBO, MLT and SRC groups over baseline and recovery days. *: significant difference between drug conditions ($p < 0.05$) within each recovery day (D1-10); +, ++, ++++: significant difference between each recovery day and the baseline day (D1) within each drug group ($p < 0.05$; $p < 0.01$; $p < 0.0001$, respectively). Sleep latencies are expressed in seconds.

Compared to baseline, PBO subjects were significantly drowsier until D6 ($p < 0.05$ to 0.0001, depending on the recovery day) and also at D9 a.m. ($p < 0.01$) and D10 p.m. ($p < 0.05$). Conversely, SRC subjects were not sleepy during the period that the drug was given (D1-D5), except on D1 and D2 p.m., where sleep latencies were reduced ($p < 0.05$). However, sleep latencies were higher under SRC than under PBO on D1 p.m. (424 ± 114 vs 74 ± 17 s, $p < 0.05$) and D2 a.m. (736 ± 155 vs 272 ± 50 s, $p < 0.05$). This stimulating effect, compared with PBO, tended to be maintained until D6 (NS). Thereafter, compared with baseline, SRC subjects were sleepier from D6, i.e. at the end of the treatment ($p < 0.05$ to 0.001 depending on the day).

Under MLT, the subjects were sleepier (sleep latencies significantly decreased, $p < 0.001$ to 0.05) than in the baseline condition over the entire recovery period during which the drug was taken (D1-D3). Subsequently, sleep latencies did not differ from baseline on D4-D5, decreased again till D8 p.m. ($p < 0.01$ to 0.05) and returned to baseline level until D10 p.m.

Wrist actigraphic measures reflected normal daytime and nighttime profiles in accordance with the time-table of the experiment (rest/test periods) in all subjects throughout the study (Fig. 4). There was no significant difference in wrist activity between drug conditions within each recovery day. Comparisons between recovery and baseline did not show any difference for PBO and MLT subjects, but with SRC, overall daytime activity was higher ($p < 0.05$) from D1 to D5, i.e. over the entire recovery period that SRC was taken. Otherwise, nighttime activity was not altered with SRC, which as indicated by the EEG measurements also showed that sleep was not fragmented under SRC.

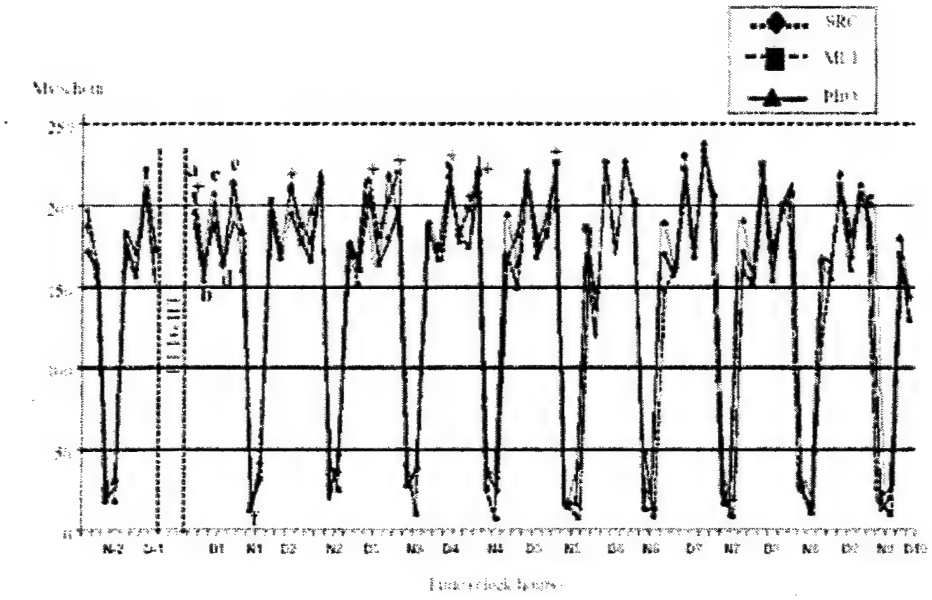

Figure 4 Wrist actigraphy (movements per hour) of PBO (triangle), MLT (square) and SRC (diamond) subjects throughout baseline and recovery nights and days. Measures reflect a normal activity profile; for example above D1 see a: wake up then breakfast; b: morning tests; c: lunch; d: afternoon tests; e: dinner then moving to the bedroom; f: night time. +: significant difference between recovery and baseline days and nights within each drug group ($p < 0.05$).

Cognitive Performance

Selected tests from the AGARD-NATO STRES Battery were used to evaluate cognitive and psychomotor performance. The tests were taken twice a day, in the morning session and the afternoon session. None of the tests produced statistical differences between the SRC, PLA or MLT conditions. Only the number of correct response per minute (throughput) are shown. As can be seen in Figure 5 learning continued to occur throughout the experiment. For the matrix task, participants had to compare the current checkerboard pattern with the one just prior to it. If it was identical, one key would be entered but if different, another would be entered. For the matrix data it looks as if the throughput for the MLT condition was slightly better than the others.

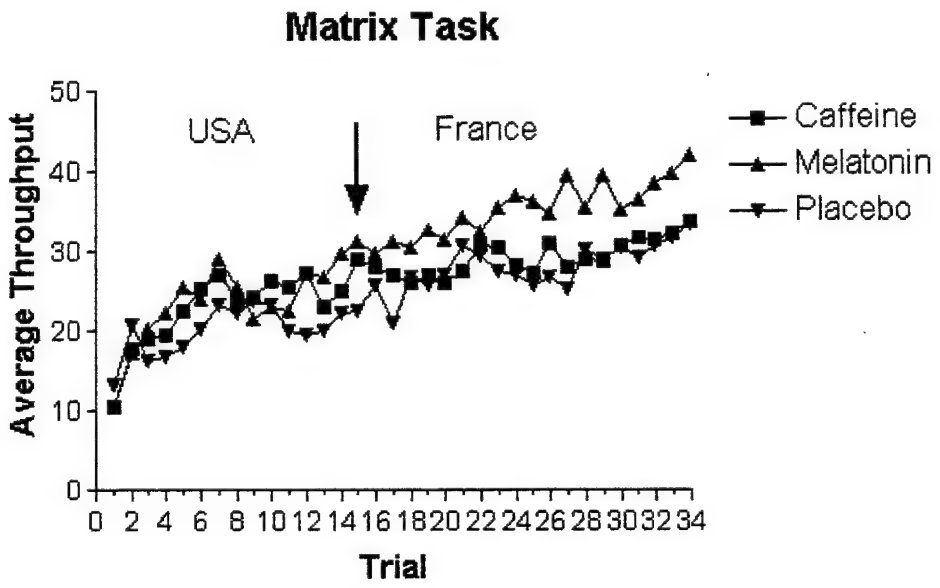

Figure 5. The Matrix performance task for the SRC, PLA and MLT groups. The arrow indicates where the trip to the French labs occurred.

Data from the Continuous Recognition test reveal the same pattern. Learning continued to occur throughout the experiment. In this test, participants had to enter a certain key if the current number on the computer screen was the same as the one just prior to it. If it was different, another key entry was made. Figure 6 suggests that the PBO condition did slightly better on this test.

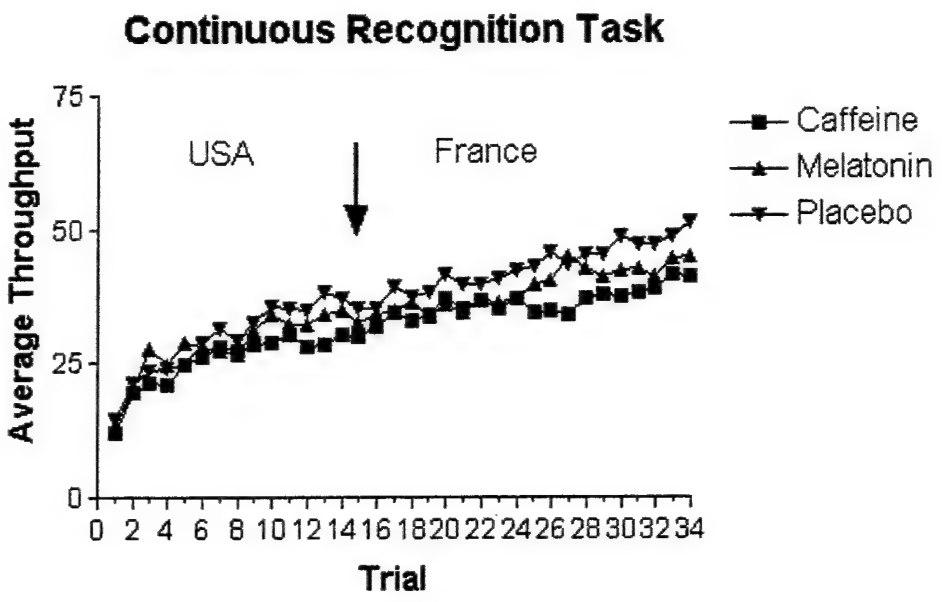

Figure 6. The Continuous Recognition data for the SRC, MLT and PLA conditions.

The Divided Attention Dual Task results are shown in Figure 7&8. The manikin data are shown in Figure 7. The manikin task requires identifying whether an object is in the left or right hand of the manikin on the screen. All the while they had to attend to an arrow at the bottom of the screen to see if the task had changed to an addition problem (Figure 8).

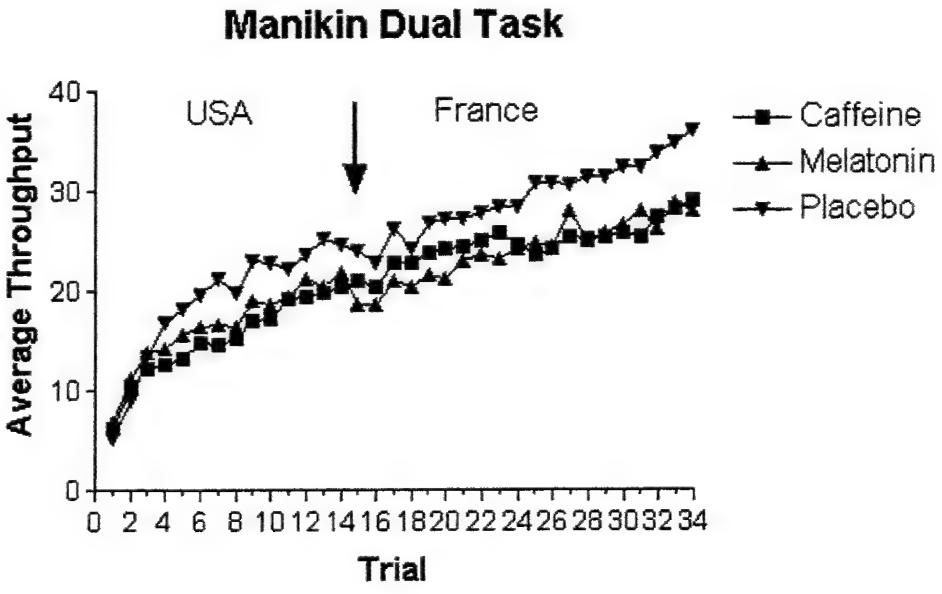

Figure 7. The Manikin component of the Divided Attention Dual task.

Again, Figure 7 seems to suggest that the PBO group did slightly better than the MLT or SRC groups but this was not significant.

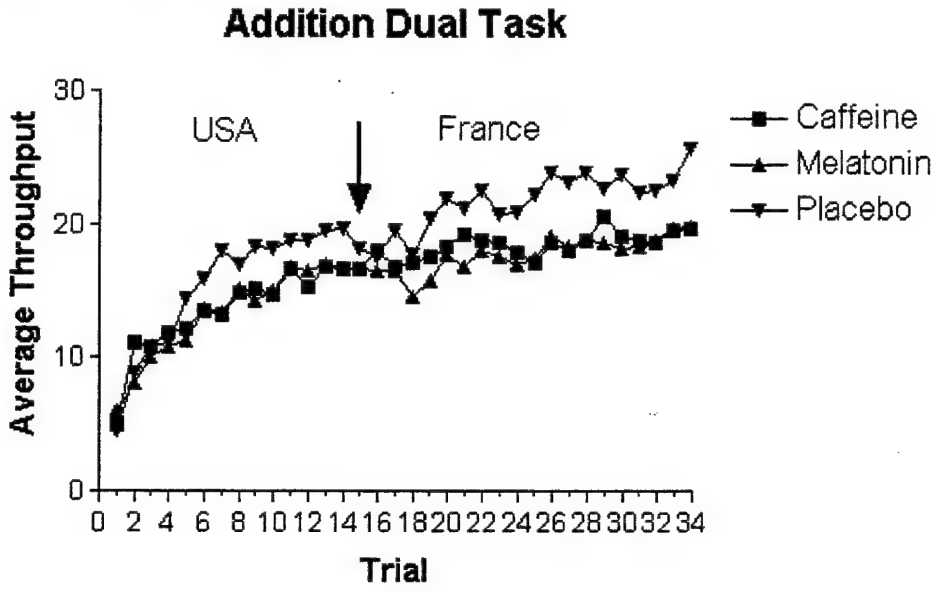

Figure 8. The Addition component of the Divided Attention Dual Task.

The remaining task was a psychomotor task, Critical tracking. The participants had to use a mouse to control a cursor on the center of the computer screen. The cursor would increasingly become more difficult to control and eventually would disappear off the screen. The RMS error score reflects how far from center the cursor is and the control losses score reflects the number of times the cursor disappears. Figure 9 shows the control losses scores for the 3 groups.

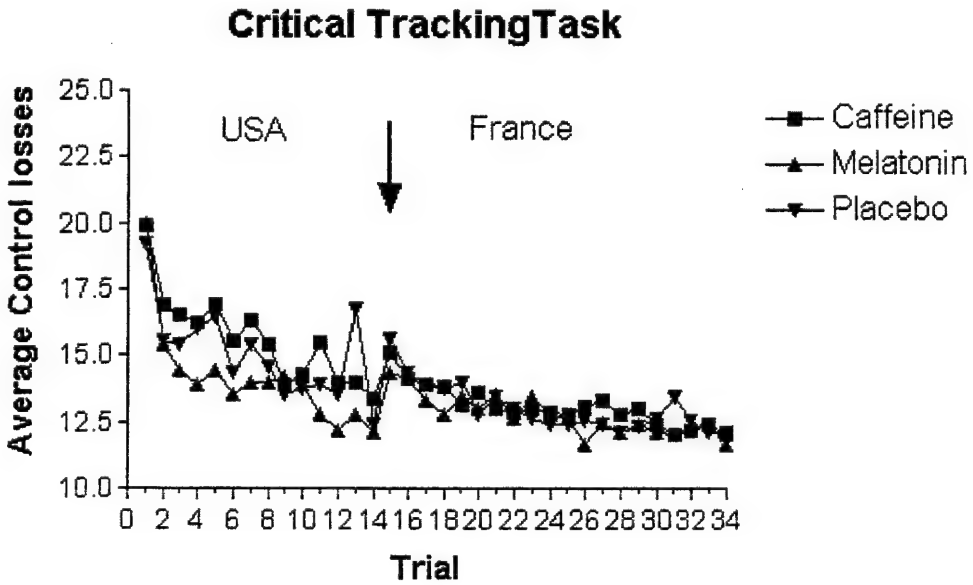

Figure 9. The results from the critical tracking task for all 3 groups

Finally, the RMS error score (Figure 10) seems to be less affected by learning than the other tests.

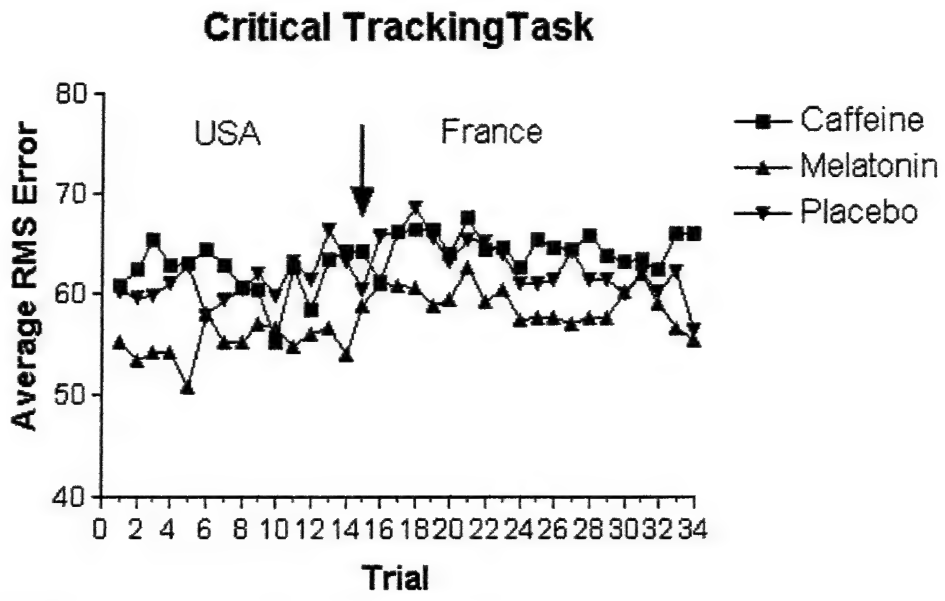

Figure 10. The RMS error scores for the critical tracking task.

Subjective measures of vigilance

As for the other tests of vigilance, there was no significant difference between the three drug groups regarding the awake/sleepy item on Bond and Lader's visual analog scale (VAS), except on D1 a.m., where MLT subjects were sleepier than the SRT group (Fig.11).

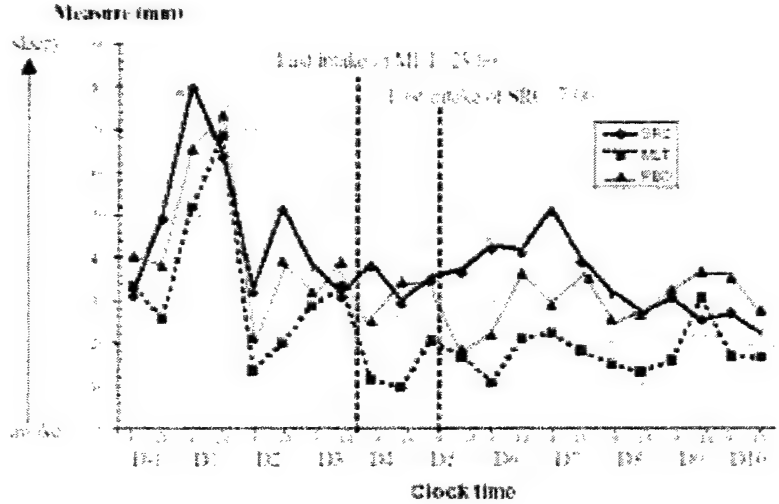

Figure 11. Subjective assessment of vigilance on Bond and Lader's visual analog scale (item awake/sleepy). * significant difference between drug conditions ($p < 0.05$) within each

recovery day (D1-10); +, ++, +++, ++++: significant difference between recovery days and the baseline day (D-1) within each drug group ($p < 0.05$; $p < 0.01$; $p < 0.001$; $p < 0.0001$, respectively). Scale is graduated in mm.

VAS confirmed the sleepiness shown by the MSLT in PBO subjects on D1 a.m. and p.m. only ($p < 0.05$ and $p < 0.01$, respectively), but not for the remaining recovery period during which subjects felt more awake on D2 a.m., D5 p.m., D6 a.m. and D8 a.m., compared with baseline ($p < 0.05$). SRC subjects felt sleepier in D1 a.m. only ($p < 0.0001$), but were less sleepy in the afternoon of D3 ($p < 0.05$), D4 and D8 ($p < 0.01$), D9 and D10 ($p < 0.001$). Under MLT, subjects felt sleepier only on D1 p.m. ($p < 0.001$), but not on D2 and D3 as shown by MSLT.

No significant differences were observed between the three drug groups within each day of the study, except for the MLT subjects who felt more sleepy during the flight ($p < 0.05$) than the two other groups of subjects who had not taken any active drug (they included the SRC subjects whose treatment began on the morning of D1). MLT subjects were also more sleepy than SRC subjects on D1 a.m. ($p < 0.05$).

Saliva melatonin

Salivary melatonin values ranged between 0.14 and 409 $pg \cdot ml^{-1}$ of saliva, with a mean value of 30 pg·ml-1, or 130 pmol·l-1 (melatonin molecular weight=232). A Student's t-test did not reveal significant differences in melatonin concentrations between male and female subjects, under the control conditions in the United States. A comparison of melatonin concentrations under control conditions among subjects less than 35 years old (n=13) and more than 35 years old (n=14) was also performed. There were no significant differences for this parameter with respect to age. Accordingly, data were processed independently of the sex or age of subjects. An ANOVA on raw data showed significant day and subject factors ($p<0.001$). The data were then assessed according to the three treatment groups (placebo n=9; melatonin n=9; slow-release caffeine n=9) and mean values were calculated for each day. An ANOVA on mean values confirmed the influence of a day factor ($p<0.001$), but not of a treatment factor. Intergroup comparisons of mean values by Student's t-test, day by day, did not reveal significant differences among treatments, as to saliva melatonin concentrations, for a given day. Intragroup comparisons, for each of the three groups, relative to the mean value calculated for the three control sessions in the United States, were performed using a paired Student's t-test (each group being its own control). The placebo group had saliva melatonin concentrations significantly higher for Day 3 ($p<0.05$), Day 4 ($p<0.05$), Day 5 ($p<0.001$) and Day 10 ($p<0.05$). For the melatonin group, mean saliva melatonin concentrations were different from mean control concentrations only for day 10 ($p<0.05$), the last day of the experiment. For the slow-release caffeine group, saliva melatonin concentrations were never significantly different from the mean control concentrations (Figure 12).

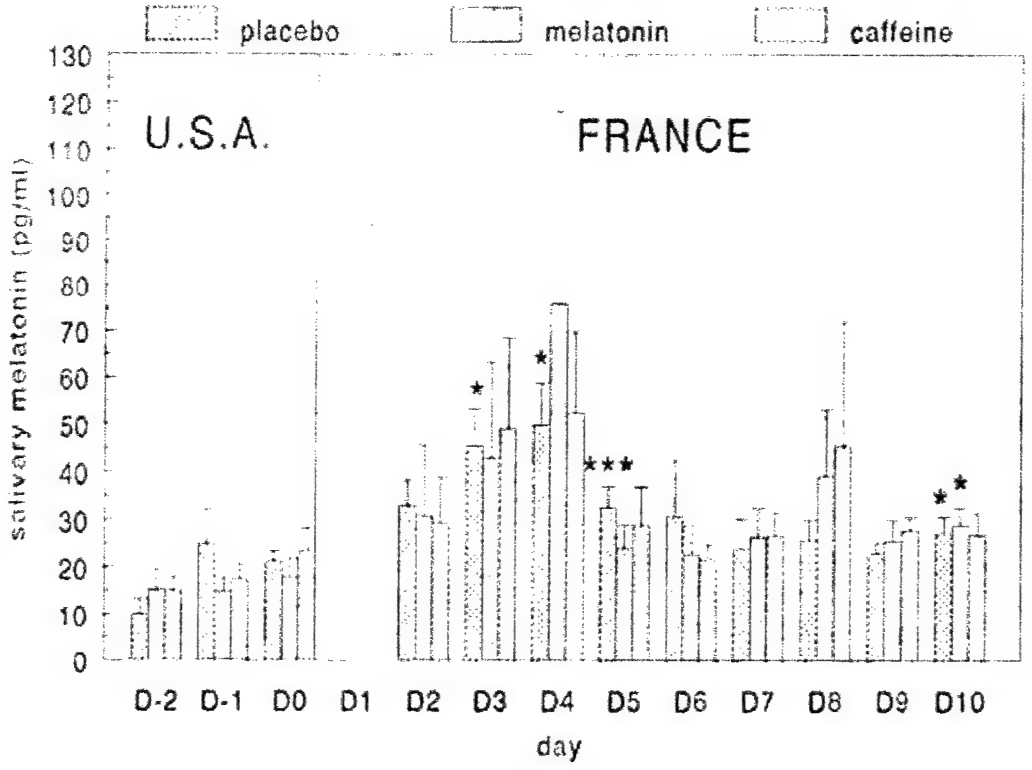

Figure 12. Salivary melatonin values across days in the USA and in France. ★ indicates a significant change from baseline.

Saliva cortisol

Cortisol values ranged between 0.2 and 27.1 $ng \cdot ml^{-1}$ of saliva, with a mean value of 3.7 $ng \cdot ml^{-1}$, or 10.2 $nmol \cdot^{-1}$ (cortisol molecular weight=362). As in the case of melatonin, a Student's t-test did not reveal significant differences in cortisol concentrations between male and female subjects, under control conditions in the United States. As for melatonin, there were no significant differences between salivary cortisol concentrations for subjects less than 35 years old (n=13) compared to those more than 35 years old (n=14) under control conditions. Accordingly, data were processed independently of the sex or age of subjects. An ANOVA on raw data showed significant day and subject factors ($p<0.001$). As for melatonin, data were analyzed according to the three treatment groups (placebo n=9, melatonin n=9, slow-release caffeine n=9) and the mean values calculated for each day. An ANOVA on mean values confirmed the influence of day factor ($p<0.001$), but not of treatment factor (Figure 13). Intergroup comparisons of mean values using Student's t-test, day by day, did not reveal significant differences among treatments, with regards to saliva cortisol concentrations, for a given day. Intragroup comparisons, for each of the three groups, relative to the mean values calculated for the three control sessions in the United States, were performed using a paired Student's t-test (each group being its own control). The placebo group had saliva cortisol concentrations

significantly lower than mean control value, from Day 2 to Day 9 included, with highest level of significance for Day 3 ($p<0.001$). For the melatonin group, mean saliva cortisol concentrations were significantly decreased from baseline concentrations, on Day 2 through Day 5, with the highest level of significance for Day 2 ($p<0.001$). For the slow-release caffeine group, the saliva cortisol concentrations (as for the melatonin group) were significantly decreased from concentrations, on Day 2 through Day 5, with a minimal value on Day 2 ($p<0.01$). Moreover, the cortisol concentrations for the slow-release caffeine group seemed to be less depressed than for the melatonin group during the first few days after arrival, though differences were not significant.

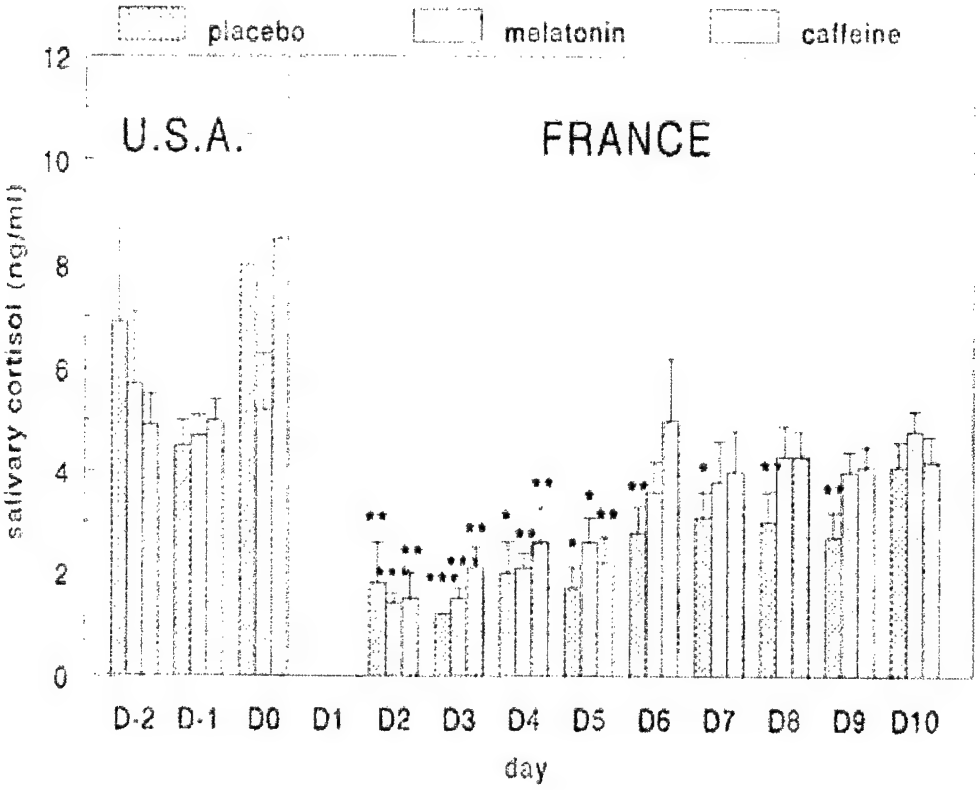

Figure 13. Salivary cortisol levels across days in the USA and days in France. *, **, ***: significant difference between recovery days and the average of the 3 control days within each drug group ($p < 0.05$; $p < 0.01$; $p < 0.001$, respectively).

Urinary 6-sulphatoxymelatonin

Measurements of the total urine produced between 11 p.m. to 7 a.m. did not indicate a diuretic effect of either the melatonin or the slow-release caffeine treatments in comparison with the placebo. The a-MT6 s concentrations have been expressed as micrograms per hour to correct for variations in urine quantity. Urinary a-MT6 s concentrations in the placebo group ranged from 0.06 to 4.70 µg·h-1 with a mean value of 1.76 ± (SEM 0.14) µg·h-1. During the period between

Day -1 and Day 3 (the period being treated by melatonin), the mean urinary a-MT6 s concentrations in the melatonin group [3.3 $\pm$ (SEM 0.5) $\mu g \cdot h\text{-}1$] were significantly higher (more than double) compared to a-MT6 s concentrations in the placebo group [1.6 $\pm$ (SEM 0.4) $\mu g \cdot h^{-1}$; $p<0.02$] and in the slow-release caffeine group [1.7 $\pm$ (SEM 0.3) $\mu g \cdot h^{-1}$; $p<0.05$].

Saliva caffeine

The maximal caffeine concentration occurred each day at noon (4 h after daily administration). At this time, mean caffeine concentrations in saliva were 2.380 $\pm$ (SEM 0.133) $\mu g \cdot ml^{-1} \cdot (70 \text{ kg body mass})^{-1}$ for the men and 6.838 $\pm$ (SEM 0.487) $\mu g \cdot ml^{-1} \cdot (70 \text{ kg body mass})^{-1}$ for the women. At 1 h before caffeine administration (23 h after the previous caffeine ingestion), i.e. at 7 a.m., the mean concentration was 0.550 $\pm$ (SEM 0.087) $\mu g \cdot ml^{-1} \cdot (70 \text{ kg body mass})^{-1}$ for the men and 1.574 $\pm$ (SEM 0.318) $\mu g \cdot ml^{-1} \cdot (70 \text{ kg body mass})^{-1}$ for the women. Thus, mean caffeine concentrations for the female subjects were 2.9 times higher compared to the male subjects. However, the same ratio of 4.3 between caffeine concentrations at 12 a.m. and 10 p.m. occurred in both male and female subjects.

DISCUSSION

To our knowledge this double-blind, controlled study is the first to have been conducted in real operational conditions. The study assessed the efficacy of a new pharmacological form of caffeine, called slow release caffeine, versus melatonin and placebo on sleep and daytime vigilance during a ten-day and nine-night recovery period following an eastbound flight across seven time zones. The action of melatonin on sleep and sleepiness under jet-lag conditions was measured objectively from EEG recordings.

Our field study combined the effects of jet-lag and sleep deprivation and this makes our results difficult to compare with those from laboratory studies. However, the main findings from our study are that MLT and SRC mitigate daytime jet-lag-related disorders. We noted actions and adverse reactions related to the pharmacodynamics of each drug: a positive effect of caffeine on daytime sleepiness with a negative effect on sleep. No alterations in recovery sleep combined with any negative effects on daytime vigilance were noted in the subjects treated with MLT.

We chose to use a 300-mg dose of SRC because this dose has been demonstrated to have positive effects on vigilance and performance during a 32-h sleep deprivation over 13 h after intake without any major side-effects (Lagarde, et al., 2000). The action of slow release caffeine (300-mg SRC) was compared to that of 5-mg melatonin (MLT) in accordance with the protocol for alleviating jet- lag proposed by Arendt (et al., 1999) when going east, take one 5-mg capsule of melatonin on the day before departure day and on departure day at approximately 1800 local time and on arrival at local bedtime (2200-2300) for 4 days (Arendt, et al., 2000). A dose above 5 mg (10 mg daily) would not have been fully cleared from the circulation after an 8-h sleep (Comperatore, Lieberman, Kirby, Adams, and Crowley, 1996) and a lower dose would have been less effective in alleviating jet-lag-related sleep disorders (Suhner, Schlagenhauf, Johnson, Tschopp, and Steffen, 1996).

Nighttime sleep and daytime sleepiness

Sleep measurements were obtained from polysomnography only in the male subjects. However, as noted in female flight attendants flying transmeridian routes (Suvanto, Prtinen, Harma, and Ilmarinen, 1992), our sleep logs revealed greater sleep disturbances in women during the 1st half of the recovery period in all treatment groups, although slow release caffeine tended to alleviate these disturbances. The sensitivity to jet-lag is also known to depend on age (Suvanto, et al., 1990). Most of the sleep parameters in our study exhibited a relatively large standard deviation, most likely due to the age range of our population (19-47 years). However, the allocation of this population into the three drug groups was designed to minimize age effects. Indeed, no age effect was observed in our three groups during recovery.

Daytime sleepiness was assessed using tests which are known to be sensitive to sleep deprivation and which are commonly employed to evaluate the effects of psychotropic drugs on vigilance in humans. Sleep latencies obtained from the MSLT reflect the alerting action of a drug (Thorpy, 1992) with or without sleep loss (Zwyghuizen-Doorenbos, Roehrs, Lipschutz, Timms, and Roth, 1990). Wrist actigraphy is an indirect but objective tool for determining the sleep-wakefulness rhythm (Lowden, and Akerstedt, 1999). The data have been found to be well correlated with EEG recordings during shift work in young and elderly subjects (Reid, and Dawson, 1999) and with sleep logs ((Lockley, et al., 1999).

Baseline sleep

We did not observe any sleep disturbances during the baseline night, indicating the absence of a first night effect (Agnew, Webb, and Williams, 1996). This was expected as our subjects spent five days and nights in Texas for synchronization before the day and night baseline period began. The sleep measures were in agreement with literature data for the range of ages of the subjects (Feinberg, 1994).

Recovery sleep and daytime sleepiness

Our subjects experienced the deleterious effects of jet-lag combined with sleep deprivation. Concerning the PBO subjects, sleep architecture was disturbed during the first recovery night with a greater duration of SWS at the expense of REM sleep (Graeber, 1989). Indeed, REM sleep tends to be predominant at the end of the night, but due to a phase advance of sleep rhythms, our PBO subjects woke up before getting the full amount of REM sleep. Hence, PBO subjects were sleepy during the first recovery daytime, in accordance with data from previous studies (Wright, Vogel, Sampson, Knapik, Patton, and Daniels, 1983). The debt of SWS and REM sleep mounted during the flight and the 1st recovery day but was totally recovered after the three following nights so that overall sleep architecture was normalized from the fifth recovery day with an absence of sleepiness after D6, as shown by MSLT. This is in accordance with the mean re-entrainment shift rate for the sleep/wake cycle which is about 1 h/day after an eastbound flight (Aschoff, Hoffman, Pohl, and Wever, 1975) or seven days for a 7-h eastbound flight as in our study. As for the sleep / wake rhythm, we also observed in our PBO subjects that the resynchronization of rhythms on D5 was complete for melatonin and partial for cortisol and central temperature (Piérard, Beaumont, Enslen, Chauffard, Tan, Reiter, Fontan, French, Coste, and Lagarde, 2001) in accordance to previous data (Haus, Halberg, Nelson, and Hillman, 1968).

Compared with placebo, melatonin (MLT) has been shown to improve subjective measures of

sleep and alertness after jet-lag (Arendt, et al., 2000). Self-reports showed that our MLT subjects fell asleep earlier and slept longer than did our PBO subjects. There is little information on the action of MLT on objective parameters of sleep. One study reported that MLT shortened sleep onset latency (SOL) and SWS duration (Zhdanova, et al., 996), without modifying REM sleep duration but sometimes lengthening it (Cajochen, et al., 1998). By contrast, in our MLT subjects, EEG showed that SOL was not shortened and that SWS was increased at the expense of REM sleep. This discrepancy could be explained in part by the high individual variability in the pharmacokinetics of melatonin, which may give rise to marked differences in sensitivity (Arendt, 1999). Moreover, the hypnotic effects of MLT on the sleep EEG are short-lived, even though melatonin levels are high at the time of sleep onset (Cajochen, et al., 1997). Lastly, our subjects were somewhat sleep deprived for about 33 hours (time between the end of the last baseline night and the beginning of the 1st recovery night) as we prohibited them from sleeping during the flight. Under jet-lag conditions, there is little evidence for a phase shifting action of melatonin on objective markers of human circadian rhythms (Samel, et al., 1991) such as sleep. MSLT data showed that our MLT subjects were sleepy until the last intake of MLT (D3), while visual analog scales showed they were sleepy up to D1. In contrast with literature data (Suhner, et al., 1996), MLT failed to maintain vigilance in our subjects. It should be borne in mind that MLT improves vigilance and alertness after jet-lag in non-sleep-deprived subjects. In our subjects, the sleepiness may have stemmed more from the sleep deprivation than a residual hypnotic effect of MLT as saliva and thus plasma levels of melatonin of our MLT subjects were comparable to those of the SRC and PBO subjects (30 pg/ml in saliva, measured at 0700) (Piérard, et al., 2001).

We observed the well known psychostimulant effect of caffeine in our SRC subjects during recovery. This alerting effect was particularly evident as the subjects could sleep for 7hr 30min and so were not sleep deprived (Linde, 1995). Nevertheless, this alerting effect was not observed on the morning of the 1st recovery day where sleep latencies and the feeling of sleepiness were higher than in baseline conditions, although motor activity was maintained. This could be explained by the kinetics of SRC (Fig. 1): the minimal efficacy level of caffeine was probably not reached at MSLT time (0900), one hour after SRC intake. As soon as the treatment was stopped, subjects were not different from the placebo group condition, consistent with the fact that the subjective (VAS) and objective (MSLT) efficacy of 300-mg SRC is lost 9-13 hours following intake.

The alerting effect of SRC seemed to induce some residual effects on recovery sleep, indicated by less SWS rebound in N1-N2 and increased nighttime wakefulness in N1 (+ ~25 min, not significant) compared with PBO and MLT subjects. Moreover, sleep logs showed that SRC subjects woke up earlier, slept less and complained of more awakenings than did the MLT group during N1. In addition, the rebound in SWS was postponed to N6 at the beginning of SRC withdrawal, whereas WASO fell on N5, the night following last intake (D5, see fig. 2) and continued to be low until the end of the study. These observations suggest that subjects were sleep-deprived under SRC. Caffeine levels in saliva samples were measured three times per day (700, 1200, 2200) throughout the study (Piérard, et al., 2001) after intake, salivary SRC was 2 µg/ml corresponding to a plasma level of 2.7 µg/ml, based on a saliva/plasma ratio of 0.74 (Sicard, Perault, Enslen, Chauffard, and Vandel, 1996). This level was higher than the plasma

level of caffeine effectiveness (2.5 μg/ml, see fig. 1), thus explaining the disturbances of sleep in the recovery period.

Although caffeine has not been shown to have any well-established direct chronobiotic properties (Winget, Soliman, Holley, and Meylor, 1994), the alerting effects of SRC on recovery sleep may have masked its chronobiotic action. Indeed, SRC recovery sleep was no more disturbed than in the baseline condition. However, SRC as well as MLT speeded up the resynchronization of cortisol and endogenous melatonin secretion by four days relative to placebo (Piérard, et al. 2001). The nighttime residual level of caffeine may have been sufficient to reduce melatonin secretion due to an antagonist action at the A2b adenosine receptors, which are present on pinealocytes (Wright, Badia, and Myers, 1997).

Saliva melatonin concentrations are a reliable indicator of pineal melatonin concentrations in the general circulation. Saliva melatonin concentrations are about one-third of the plasma values. Nocturnal plasma concentration are about 3 to 10 times greater than during daytime. The peak saliva melatonin concentration, without pharmacological treatment, ranges between 45 and 200 pmol·l-1, with a mean value of 106 (SEM 17) pmol·l-1. In the current study, the peak saliva melatonin concentration without treatment (placebo group) occurred on day 5 ($P<0.001$), and was 140 (SEM 19) pmol·l-1. Thus, the current results are in accordance with those of earlier studies.

Intergroup comparisons did not reveal significant differences between the three treatment groups for saliva melatonin concentrations. The subjects who had received exogenous melatonin had saliva melatonin values similar to subjects who had received the placebo or the slow release caffeine upon awakening. This was probably due to the short melatonin half-life, which is less than 1 h (Comperatore et al. 1996). Since melatonin was administered before bedtime (11 p.m., France time) and saliva was collected at 7 a.m. the following morning, it was to be expected that no or little exogenous melatonin would still be present in the blood 8 h after its administration. In contrast, urinary a-6MT s concentration, the main hepatic melatonin metabolite, was higher in the urine of the melatonin group in comparison with other groups. In normal human subjects, the excretion rate is about 1.1 μg·h-1 during the night; in the current study the excretion rate was 1.6 (SEM 0.4) μg·h-1.

Except for the last day of the experiment (Day 10), the only saliva melatonin concentrations significantly different from control concentrations were those of the placebo group from Day 3 to Day 5. The acrophase of blood melatonin concentrations normally occurs at about 3 a.m. After transmeridian flight, the highest saliva melatonin concentration occurred between Day 3 and day 5, when samples were collected at 7 a.m. (French time). For Day 3, a 4 h shift apparently persisted, and consequently, in this case of a 7 h lag in time, a 3 h resynchronization seemed to have occurred. The mean re-entrainment shift rate for circadian rhythms after a westbound flight is 57 min·day-1 (Aschoff et al. 1975), or roughly 3 h after 3 days. Moreover, after a 9 h phase advance, induced by adjusting the lighting conditions in the laboratory, 5 days are required to resynchronize a subject's melatonin secretion profile (and awake-sleep cycle, internal temperature rhythms and psychomotor performance). Our results are quite in accordance with these works.

Post-flight saliva melatonin concentrations in the melatonin and the slow-release caffeine groups never differed from United States control concentrations (Day -2 to Day 0). We, however, observed a statistically insignificant tendency to an increase of mean concentrations until Day 4. Melatonin given later in the afternoon before leaving, seemed to induce on the same day (Day -1) a 4 h phase advance in melatonin secretion. Melatonin administration on the following days seemed to stabilize this phase advance. However, Arendt et al. (1997) have shown that exogenous melatonin administration is more effective in terms of sleep-awake cycle resynchronization than on melatonin rhythm resynchronization. Other authors have shown that light has a more effective re-entrainment effect than melatonin administration on melatonin secretion itself.

It is likely that the melatonin and the slow-release caffeine groups were resynchronized earlier than were the placebo group subjects, as regards their melatonin secretion rhythm. As mentioned earlier, caffeine has not been shown to have any direct well-established chronobiotic properties. The residual caffeine concentrations (about 0.5 μg·ml-1 of saliva) may have been sufficient to reduce melatonin secretion. The reasons why saliva caffeine concentrations were higher in the women than in the men (after adjustments for body mass) remain unclear. While the caffeine concentration was about three times higher in the women than in the men, caffeine seemed to be metabolized at the same rate in the men as in the women. Saliva melatonin concentrations in the placebo and the melatonin groups differed significantly from control values on day 10 ($P<0.05$). The reason for this minor difference is not apparent.

Collections of urine produced overnight confirmed the absence of a diuretic effect after the administration of either exogenous melatonin or after slow release caffeine. This latter result is in accordance with laboratory data concerning the lack of secondary effects of this new galenic form of caffeine.

The saliva cortisol peak is normally about 16.5 nmol·l-1. During the present study, the saliva cortisol peak was 16.3 (SEM 1.4) nmol·l-1, at 7 a.m. under control conditions in the United States. Thus, these results are in obvious good agreement with those previously published. Our results confirm first that jet lag induces desynchronization of cortisol secretion and secondly that melatonin administration in humans may hasten the resynchronization of cortisol rhythms following an abrupt phase shift. Moreover, our results show that the resynchronization of the melatonin rhythm following a time zone change is more rapid than that of the cortisol rhythm. Indeed, the cortisol secretion rhythm appears to have been resynchronized only on Day 10 in the placebo group (instead of Day 6 for melatonin secretion), and on Day 6 for the melatonin and the slow-release caffeine group (instead of Day 2 for melatonin secretion for both groups). Accordingly, subjects treated with melatonin or slow-release caffeine seem to have been resynchronized 4 days earlier than the placebo group subjects, as regards their melatonin and cortisol secretion rhythms with the doses used.

CONCLUSION

Our study compared for the first time in field conditions, the effects of the administration of melatonin with a new slow release caffeine formulation on recovery sleep and vigilance after an eastbound jet-lag combined with sleep deprivation. Although melatonin and to a lesser extent slow release caffeine hastened resynchronization of biological rhythms, melatonin had little action on recovery sleep due to the sleep deprivation that was combined with the jet-lag in our subjects. However, slow release caffeine did alter sleep patterns due to its prolonged alerting action. In contrast with slow release caffeine, melatonin failed to alleviate daytime sleepiness. We concluded that for military operations including jet-lag and sleep deprivation, slow release caffeine may be of value for reducing sleepiness for a few days. Further studies on jet-lag without concomitant sleep deprivation will be required to evaluate fully the effects of slow release caffeine compared to melatonin on recovery sleep and vigilance following an eastbound flight.

The results obtained during this experiment firstly confirm previous work concerning the effects of jet lag on the secretion of melatonin and cortisol, and secondly support the effectiveness of melatonin in resynchronization of hormone rhythms. Moreover, our results suggest that caffeine also has chronobiotic properties, probably enhanced because of its slow-release formulation.

In conclusion, slow-release caffeine seems to be at least as effective as exogenous melatonin for re-establishing melatonin and cortisol secretion rhythms and probably endogenous clock resynchronization after time changes following transmeridian travel.

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